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Growth and Fecundity of *Pomacea patula* (Caenogastropoda: Ampullariidae) When Fed on Gel Diets of *Scenedesmus incrassatulus* (Chlorophyceae)

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Abstract. The commercial capture of *Pomacea patula* ("tegogolo" or "apple snail") in Catemaco Lake, Veracruz, México, has decreased considerably in recent years. Its conservation and continued use as a regional economic resource is threatened, because little biological-ecological information is known for the species and measures regulating the intensity of its exploitation are deficient. As a contribution to the biological knowledge of this mollusk, the present study evaluates the effect of diet on *P. patula* growth and reproduction in laboratory. Two types of diet were applied, each gelled with 8% cornstarch, and containing varying proportions of the microalga *Scenedesmus incrassatulus* (Chlorophyceae). Diet 1 contained 100% microalgae, whereas in diets 2, 3, and 4, *S. incrassatulus* was gradually substituted with balanced trout food in proportions of 10%, 20%, and 40% dry weight, respectively. After twelve months of observation, no significant differences in growth, as measured by size and weight, were noted ($P < 0.05$). All experimental mollusk populations reached sexual maturity at six months of age, and fecundity was higher when the proportion of balanced food in the diet was increased. Survival during the observation period fluctuated between 70% and 85% regardless of diet.

INTRODUCTION

Ampullariidae are amphibious freshwater snails; *Pomacea* Perry, 1810, is the most representative genus of the family and is commonly known as the "apple snail." This mollusk is native to tropical regions of the American continent, from which it has dispersed to other parts of the world; this has been partially due to its culinary value as well as to its importance to aquarium owners (Cowie, 1993). The natural distribution of *Pomacea patula* Baker, 1922, in Mexico is limited to Catemaco Lake (Naranjo & Cubas, 1985), a place where in the 1980s, total commercial capture recorded surpassed 5000 tons, maintaining an average of 502 tons/yr (De la Lanza & García, 1995). This volume began to drop significantly in the early 1990s, the probable result of over-exploitation coupled with variation in lake water volume due to operation of the Chilapan hydroelectric plant (Pérez & Orozco, 1992) and pollution from pesticides used in nearby agricultural areas (Calderón, 1997). The permanence and sustainable use of this resource is now threatened due to unregulated commercial exploitation and the fact that the mollusk's biology is not well understood; protection strategies that could be applied to the recovery of wild populations are yet to be implemented.

Aquaculture can contribute to the rehabilitation of this resource through controlled production of young for repopulation, a quite feasible process due to certain characteristics of the species such as reproductive potential, growth rate, and direct development, which make it a potentially cultivable species in Mexico (Valdés & Aguilera, 1986). Regionally, some basic research has been done on the biology of the genus *Pomacea* (Rangel, 1984; Osorio, 1987), along with studies on preliminary cultivation of the species for aquacultural purposes, with vegetables and aquatic macrophytes as food (Ontiveros, 1989; Martínez, 1989; Amaya & Godínez, 1994; Lagunes, 1997). However, most of this research consists of isolated efforts, the results of which are not found in scientific papers; little published information is available that might contribute to designing an aquaculture program for this species in Mexico despite its undoubted biological and socioeconomic importance, especially for the fishing industry (Torres & Rojas, 1995; Negrete, 1997).

One of the most important factors limiting the growth potential of this snail is food. Members of the Ampullariidae have three types of food that are not mutually exclusive: microphages, zoophages, and macrophytophages (Estebenet, 1995). However, the specific nutritional requirements of *P. patula* are largely unknown; analysis of the contents of the first anterior third of the digestive tract of wild specimens reveals a predominance of benthonic photosynthesizing microorganisms, including settled

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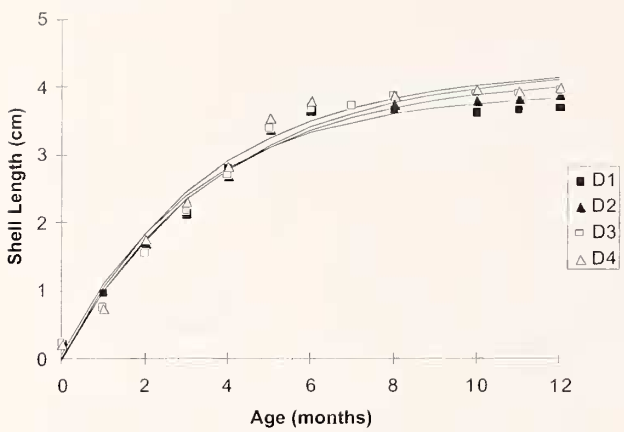

Figure 1. Fit of Von Bertalanffy growth model to shell length increase (cm) of *Pomacea patula* when fed four diets containing the microalga *Scenedesmus incrassatulus*.

Figure 2. Fit of logistic growth model to weight increase (g) of *Pomacea patula* when fed four diets that contain the microalga *Scenedesmus incrassatulus*.

Chlorophyceae and Bacillariophyceae microalgae, among which the genera *Chlorella, Scenedesmus,* and *Ankistrodesmus* stand out along with some cyanobacteria, free-living ciliates, rotifers, fish eggs, insect remains, and aquatic macrophytes, as well as abundant sand particles (Espinosa-Chávez & Martínez-Jerónimo, unpublished). These data corroborate *Pomacea*'s frequently recognized omnivorousness, which has been the basis of much feeding research such as that of Mendoza et al. (1999), who demonstrated that *P. bridgesi* grows better with diets that incorporate animal protein sources (provided by fishmeal) rather than with those that feature only vegetable protein (from aquatic macrophytes). Analyses of the stomach contents of *P. canaliculata* have revealed, among other material, insects, crustaceans, fish eggs and remnants, although it is frequently recognized as a species of microphytophagous habits (Estebenet, 1995). In captivity, however, it has been observed to ingest dead animals even when vegetable matter is present, suggesting a need for animal protein (Estebenet & Cazzaniga, 1992).

The present study evaluates how "tegogolo" growth and reproduction are affected by the application of two types of diet, one consisting exclusively of the microalga *Scenedesmus incrassatulus* and the other consisting of this microalga mixed with varying amounts of balanced rainbow trout food.

MATERIALS AND METHODS

The test organisms were obtained from manually collected egg masses from the shoreline of Catemaco Lake in Veracruz, Mexico. Egg masses were transported in individual glass containers that also worked as a humid incubator, shielding the eggs against dehydration. In laboratory, egg masses were incubated at 25°C with environmental light; eclosion began on the sixth day of incubation. Fifty recently hatched snails (0–48 hr old) were distributed randomly in each experimental unit, these units consisting of 30-liter glass containers that had air bubbles to oxygenate the water and a top to prevent the organisms from escaping.

The green microalgae *Scenedesmus incrassatulus* was grown in the Bold's Basal Medium (Stein, 1973) in 40-liter tubular polyethylene systems following the methodology suggested by Martínez-Jerónimo & Espinosa-Chávez (1994). Microalgal biomass was separated through sedimentation in refrigerated conditions of exponentially grown cultures of *Scenedesmus,* allowing us to obtain a concentrate with 6–8% dry material that was used to pre-

Table 1

Values for coefficients of the Von Bertalanffy growth equation for size increase of *Pomacea patula* when fed different diets over a 12-month period. Average values and confidence intervals ($P = 0.05$).

	L_{max}	K	t_0	r^{2*}
Diet 1	3.9043 ± 0.4944	0.3123 ± 0.1337	−0.0492 ± 0.0510	0.9733
Diet 2	4.1190 ± 0.5700	0.2775 ± 0.1207	−0.0100 ± 0.0533	0.9791
Diet 3	4.2648 ± 0.5932	0.265 ± 0.1121	0.0131 ± 0.0524	0.9795
Diet 4	4.2382 ± 0.5406	0.289 ± 0.1174	0.0154 ± 0.0490	0.9776

* Coefficient of determination.

Table 2

Values for coefficients of the logistic growth equation for weight increase of *Pomacea patula* when fed different diets over a 12-month period. Average values and confidence intervals ($P = 0.05$).

	K	b	a	r^{2*}
Diet 1	13.3274 ± 0.3571	1.1989 ± 0.1831	4.858 ± 0.7287	0.9890
Diet 2	14.6890 ± 0.3775	1.0836 ± 0.1502	4.8228 ± 0.6436	0.9897
Diet 3	16.0056 ± 0.445	1.0700 ± 0.1364	4.9034 ± 0.5997	0.9936
Diet 4	16.4287 ± 0.4398	1.0802 ± 0.1317	4.7275 ± 0.5477	0.9928

* Coefficient of determination.

pare the different diets. The treatments applied represented a gradient in *S. incrassatulus* content that included 100% concentrations of microalgae (D1) and a gradual substitution with formulated rainbow trout feed (growth formula with 38% protein and 10% lipids), in proportions of 10% (D2), 20% (D3), and 40% (D4).

In order to facilitate administration and consumption, the diets were gelled through the addition of 8% cornstarch. Adequate gel texture was achieved by microwaving it at 75°C for one minute (Charley, 1995) and then letting it cool at room temperature. At this point, gels were cut into 3 mm-thick slices that were supplied to snails *ad libitum*. The growth experiments were continued over a 12-month period, with monthly determinations of shell length and individual weight for all individuals. Once reproduction began, the egg masses produced were removed daily for measurement and weight; observation of reproduction was carried out from the time it began until thirteen months.

RESULTS

Figure 1 shows growth tendencies in terms of total shell length recorded for *P. patula* with the four diets tested. After 12 months of experimental analysis, it was determined that increases in shell length can be described using Von Bertalanffy's growth model, defined by this equation:

$$L = L_{\max}(1 - e^{-k(t-t_0)})$$

Table 3

Average values and descriptive statistical parameters for the weight of egg masses produced during seven months by *Pomacea patula* when fed different diets.

	Mean	s	n	Confidence Interval*	Minimum	Maximum
Diet 1	3.26	1.28	424	± 0.12	1.02	8.36
Diet 2	3.63	1.55	374	± 0.16	0.24	10.07
Diet 3	3.78	1.76	325	± 0.19	0.40	9.70
Diet 4	4.02	1.93	376	± 0.20	0.40	9.54

* $P = 0.05$

where L_{max} represents maximum possible size under the experimental conditions applied, and K the growth rate. Covariance analysis as applied to the comparison of corresponding growth coefficients shows that no statistically significant differences exist among the treatments applied ($P > 0.05$). In accordance with the determined equations, theoretical curves were constructed (Figure 1): parameters of the equations obtained appear in Table 1. The highest value for growth coefficient K corresponded to Diet 1, while the longest maximum length (L_{max}) to Diet 3.

Monthly increases in the average weight of organisms fed the four diets are shown in Figure 2. They follow a sigmoid tendency that was adjusted to the logistic equation described thus:

$$W = \frac{K}{1 + e^{a-bt}}$$

For the fittings applied to this equation, the value of ***K*** represents maximum possible weight, whereas ***b*** represents the growth rate in terms of weight. The covariance analysis applied to these growth rates reveals that there were no significant differences in this parameter for the four treatments applied ($P > 0.05$). Table 2 shows values for this model's coefficients in accordance with the four diets. Theoretical curves calculated with these models are shown in Figure 2.

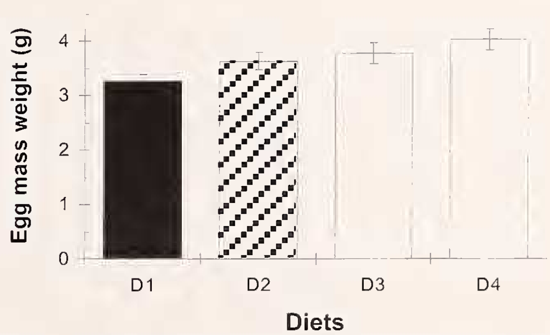

Figure 3. Comparison intervals for average weight of egg masses oviposited by *Pomacea patula* when fed four different diets. The non-overlapping limits of intervals indicate significant differences ($P = 0.05$).

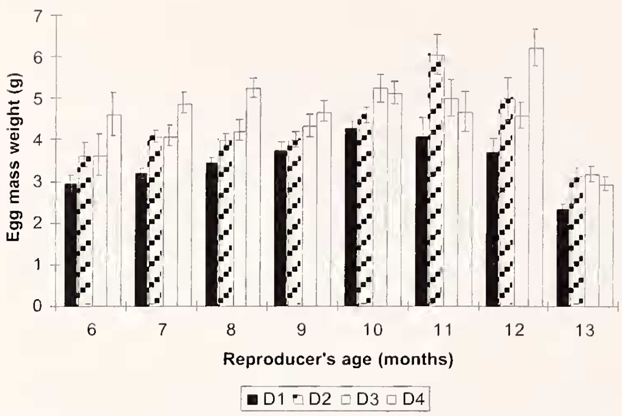

Figure 4. Average monthly weight and confidence interval ($P = 0.05$) of egg masses produced by *Pomacea patula* when fed four diets containing different amounts of the microalga *Scenedesmus incrassatulus.*

Oviposition began in the sixth month regardless of diet, and this stage coincided with the tendency for growth stabilization in terms of both length and weight (see Figures 1 and 2). Average data from all egg masses produced with the four treatments up to the age of 13 months are shown in Table 3. Analysis of variance (ANOVA) as applied to these data reveals that diet had a significant effect ($P < 0.001$), the Tukey test ($P = 0.05$) showing that compared to all other diets, organisms fed D1 produced egg masses of significantly lower average weight. There are, however, no significant differences between egg masses produced by snails that consumed diets D2 and D3, the same being true for D3 and D4 ($P = 0.05$); Figure 3 shows these comparisons.

Average weight of oviposited egg masses each month was averaged, the data appearing in Figure 4, in which it is observable that of all diets tested, egg masses were largest during the intermediate reproductive period, as both the first (early reproduction) and last (final observations, when organisms were 13 months old) were comparatively smaller.

Finally, survival after 13 months varied between 70% and 85% and was not significantly influenced by diet ($P > 0.05$).

DISCUSSION

The results observed in *P. patula* growth and reproduction with the four diets are consistent with the omnivorousness of this species as well as the feasibility of growing it with a diet consisting exclusively of microalgae; this would mean a reduction in operating costs for commercial growers, because formulated fish food costs quite a bit more. The virtues of vegetable diets have been reported by Sharfstein & Steinman (2001), who detected higher growth and survival in juvenile *P. paludosa* when fed a natural association of *Utricularia sp.* and periphyton. Similarly, Estebenet & Cazzaniga (1992) fed *P. canaliculata* juveniles and adults diets based on lettuce leaves, fish feed, dog feed, and wheat germ, creating a food that thus contained various animal and vegetable components, and found no significant differences in growth. In the present study, it was not possible to determine significant differences in length and weight increases with the four diets applied.

As to how reproduction was affected, *P. patula* made efficient use of alimentary components of a carnivore diet, represented in this case by protein and lipids of animal origin that were incorporated into formulated trout feed, as the weight of egg masses produced during the reproductive phase was directly related to the proportion of these components in snail food; this was particularly clear in the case of D3 and D4, which contained the highest amounts of formulated trout food. This observation is consistent with the results obtained by Mendoza et al. (1999), who determined that diets based on a higher proportion of fishmeal than *Pistia sp.* (as a source of vegetable protein) produced better results in terms of *P. brid-*

Table 4

Comparative growth of the "apple snail" *Pomacea patula* fed with different diets. Values for average weekly increases in weight and length are shown, as determined for each of the studies mentioned.

Species	Feed	weight (g)	length (cm)	Authors
P. patula	*Ipomea aquatica*	0.046	n. a.	Lagunes, 1997
P. patula	*Ipomea aquatica*	0.072	n. a.	Asiain & Olguín, 1995
P. patula	Alfalfa	0.056	0.137	Martinez, 1989
P. patula	*S. incrassatulus* and trout feed	0.35	0.174	This study
P. canaliculata	Cabbage and carp feed	0.0386	n. a.	Kobayashi & Fujio, 1993
P. flagellata	Chicken feed	0.294	0.161	Alonzo, 1984
P. flagellata	*Pistia sp.*	n. a.	0.132	Ontiveros, 1989
P. bridgesi	Fish feed	n. a.	0.175	Benavides, 1994
P. bridgesi	*Pistia sp.* and fishmeal	n. a.	0.345	Mendoza *et al.*, 1999
P. paludosa	Carp feed	n. a.	0.150	Ferrer *et al.*, 1991

n. a.: no data available.

gesi growth and food conversion rate. These results do not corroborate the macrophytophagia usually recognized for *Pomacea* (Estebenet, 1995).

Finally, Table 4 sums up all available information on weight and size increases for several species of *Pomacea*, illustrating contrasts with the results obtained in the present research; it can be observed that with all diets studied herein, average weekly increase in weight was higher than that recorded by other authors, the most similar results being obtained when *P. flagellata* was fed formulated bird feed (Alonzo, 1984). In terms of average weekly size increase, only Mendoza et al. (1999) reported a higher figure.

Results obtained with the four tested diets lead to the conclusion that *P. patula* can be cultivated with an exclusively vegetable diet without compromising growth, although fecundity is favored by the incorporation of an animal protein source. It is also clear that feeding the "tegogolo" only the microalga *Scenedesmus incrassatulus* is sufficient to complete the species' life cycle under controlled conditions, a promising discovery from the viewpoint of aquaculture.

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